International Research Journal of Natural and Applied Sciences

ISSN: (2349-4077)

Impact Factor 5.46 Volume 5, Issue 2, February 2018

Website- www.aarf.asia, **Email** : editor@aarf.asia , editoraarf@gmail.com

An analysis of the Antheraea mylitta hemolymph's to biochemical.

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ABSTRACT

The purpose of the research on the "Biochemical analysis of Tasar silkworm haemolymph" is to find basic strategies for enhancing silk performance characteristics that are required to create rural livelihood jobs and generate foreign income. Mylitta antherae The life cycle of the holometabolous insect Drury includes the phases of eggs, larva, pupa, cocoon, and adult. There are five larval instars, and they feed on a variety of secondary host plants in addition to the principal host plants including Arjun (Terminalia arjuna), Asan (Terminalia tomentosa), and Sal (Shorearobusta). The fifth instar larva weaves the silk around the leaves it has just devoured, creating the cocoon from which Tasar silk is reeled. The larvae feed voraciously on the leaves of their host plants. Useful silk proteins are produced by silkworms as a biofactory. These natural polymers are biodegradable, include reactive functional groups, and may be linked to other polymers to be employed in customised delivery systems.

Keywords: **Haemolymph; Protein; Larvae; Silkworm, Tasar silk.**

INTRODUCTION

The principal source of silk for the global market is the Asian area. Silk is a traditional agricultural commodity. China exports the most silk to the rest of the globe of any silk-producing nation. However, there are currently noticeable changes in the production patterns of the Asian nations that produce silk. The level of silk production in the region's advanced sericulture nations, such JAPAN and the REPUBLIC OF KOREA, has appeared to be irreversibly declining. Other nations have also seen the potential advantages of sericulture, including increased foreign exchange revenues and the reduction of rural poverty through job and income-generating activities. several of the region's emerging nations. The presence of different polar functional groups in silk, as in other widely used biomedical textiles like polyester, may improve the absorption of antibiotics. Silk is a biodegradable, environmentally benign polymer with superior moisture-absorbing and -deabsorbing capabilities. The two proteins sericin and fibroin work together to form the silk fibre, which is created from soluble protein found in the haemolymph. Many biochemical components found in haemolymph are used as starting points for the production of sericin and fibroin. Recently, research on sericin and fibroin in the area of drug delivery systems has been conducted. Fibroin has beneficial wound healing qualities and has been employed in a variety of biomedical applications. Sericin-coated powders are used in chewing gum, nail polish, wound protection films, and cosmetics for dermatitis. A biomaterial having anticoagulant characteristics is silk protein. A type of biological substance called fibroin protein is utilised in artificial skin and other medical procedures. As a result, humans have long employed the silk proteins released by Tasar silkworms in biological purposes. Tasar silk therefore plays a significant role in the textile sector, environmental protection, management of wildlife, biomedical uses, medicines, and generating foreign exchange earnings. Taking into account the importance of silkworms, The "Biochemical Analysis of Tasar Silkworm Hemolymph" study is currently being conducted. Silk is a type of natural protein fibre that may be weaved into clothing. Fibroin makes up the majority of the protein fibre that makes up silk, which is generated by some insect larvae to create cocoons. The most well-known sort of silk is made from the cocoons of domesticated mulberry silkworm bombyx mori larvae that have been raised in captivity. The triangular prism-like structure of the silk fibre, which enables silk material to refract incoming light at different angles and produce diverse hues, is what gives silk its shimmering look. Several other insects can create silk, but historically, only moth caterpillars' silk has been utilised to make textiles. Other silks with molecular differences have been the subject of some investigation. Since silk has a lower density than cotton, wool, or nylon, it is more able to absorb moisture than other materials without feeling damp. Silk can absorb up to a third of its own weight in moisture without feeling damp.

Silk manufacture:

A continuous-filament fibre known as silk is made up of the protein fibroin, which is generated by two salivary glands in each larva's head, and a glue known as sericin that holds the two filaments together. By soaking the cocoons in hot water to dissolve the sericin, the silk fibres become loose and are prepared for reeling.

Property of Silk:

Silk is a preferred fabric for clothing due to its distinctive qualities. Silk is the most opulent fabric, the most cosy fabric, the most absorbent fabric (equivalent to wool), the best fabric for drape, the best fabric for colour, capable of the highest lustre, possessing the nicest "hand", etc. These are a few of the elements that increase the fabric's appeal. The fabric is warm in the winter and cool in the summer.

Physical properties:

A protein by the name of fibroin makes up 80% of the silk fibre, while sericin, often known as silk gum, makes up 20%. Since silk is a fibre with excellent tensile strength, it can sustain heavy pulling forces. The strongest natural fibre is silk, which also has a moderate resistance to abrasion. The continuous length of the fibre is mostly responsible for the thrown yarns' strength. Although robust, spun silk yarn is weaker than thrown silk filament yarns. Silk fibre may be stretched between 1/7 and 1/5 of its original length without breaking since it is an elastic fibre. Although it progressively loses some of its flexibility, it usually grows back to its previous size. In turn, the cloth would be more comfortable to wear since it would droop and bind less. Silk has remarkable durability thanks to its flexibility and suppleness, which are complemented by its elasticity and sturdiness. Silk is a protein fibre that conducts heat poorly, much like wool. Silk is therefore appropriate for cold clothing. Silk textiles offer high absorption due to their protein composition. Even in hot environments, silk clothing is pleasant due to its ability to absorb moisture. Silk-based fabrics are cool in the summer and cosy in the winter. The average moisture absorption capacity of silk fibre is 11 percent of its weight, however the range can be as wide as 30 percent. This characteristic plays a significant role in silk's simplicity in printing and dyeing. Silk fabric's smooth surface prevents it from drawing dirt. Washing or dry cleaning may simply get rid of the filth that accumulates. The dry cleaning of silk clothing is frequently advised. Always use a mild soap and avoid using a washing machine with a lot of agitation when washing silk materials. Silk is prone to water stains, however repeated washing or dry cleaning will restore the fabric's look. Only typical shrinking affects silk materials, and ironing can restore them. Fabrics with a crepe appearance shrink significantly when washed, but a thorough ironing process with a fairly hot iron will bring the cloth back to its previous size. Silk is heat-sensitive and starts to break down about 330°F (165°C). Thus, the silk textiles must be ironed when moist. Sunlight exposure causes the silk fabric to deteriorate. Less degummed silk is more light-resistant than raw silk. Silks won't mildew unless they are exposed to extremely humid temperatures or are left moist for an extended period of time. The larvae of carpet beetles, clothes moths, or both can eat silk. Silk textiles lose strength and become yellow in the sun and perspiration. Staining results from the degradation of the silk itself and the colour being impacted. After each use, clothing that is worn close to the skin should be cleaned or washed.

Chemical properties.

As like wool, chlorine bleaches like sodium hypochlorite degrade silk. For silk, though, you can use sodium perborate or a moderate hydrogen peroxide bleach. Alkalis may not affect silk as readily as they do wool, although high concentrations and temperatures can still cause harm. Thus, it is advised to use a light soap or detergent in warm water. Silk will disintegrate in concentrated mineral acids more quickly than wool. Silk is unaffected by organic acids. Silk has an excellent affinity for colours because of its high solubility. Under normal circumstances, dyed silk is colorfast, but its level of light resistance is subpar.

Essential Proteins Found in Silk :

- **Fibroin and Sericin**

  The beta sheet antiparallel layers that make up the fibroin protein. The recurring amino acid sequence (Gly-Ser-Gly-Ala-Gly-Ala) makes up the majority of its fundamental structure. The sheets may be packed tightly due to the high glycine (and to a lesser degree, alanine) content, which contributes to silk's stiff structure that cannot be stretched (tensile strength). It is a material having uses in a variety of fields, including biomedicine and the production of textiles, thanks to its combination of stiffness and toughness. Three structures known as silk I, II, and III are known to form as fibroin arranges itself. Fibroin exists naturally in the form of Silk I, which is produced by the silk glands of Bombyx mori. The arrangement of fibroin molecules in spun silk—which is stronger and frequently employed in a variety of commercial applications—is known as silk II. A recently identified fibroin structure is called Silk III. mostly occurs at an interface in fibroin solution, when silk III is produced and Silkworm body (spun silk gland), pod (pod shell), and silk are processed to produce sericin. Sericin contains 18 different types of amino acids, with serine and aspartate having the largest concentrations. Additionally, it contains other essential amino acids for human body. It is a top-notch protein. Due to serine's hydrophilic lateral group, which has a water absorption rate 50 times greater than glycerine, which makes up around one-third of the amino acids in sericin. Sericin is a highly effective moisture absorber and preservative when used as a cosmetics' base ingredient. In order to protect the water in skin and prevent damage to skin cutin, sericin protein can produce a film on the surface of skin and hair. Serine, one of the most significant amino acids among the NMF, is found in sericin, which is great at preserving moisture. Additionally, it works effectively in preventing the production of active oxygen, which weakens the skin's surface defences, and tyrosinase, which generates freckles on the skin. It is gaining popularity as a skin care component since it is mild on the skin and works well as a moisturiser, protector, and whitener. When used with the Folin-Ciocalteau reagent, which is composed of sodium tungstate, molybdate, and phosphate, the phenolic group of tyrosine and tryptophan residues (amino acids) in a protein would generate a blue purple colour complex with maximal absorption in the area of 660 nm wavelength. As a result, the presence of these aromatic amino acids affects the colour intensity, which varies for various proteins. Because of its low cost, high purity, and easy availability, bovine serum albumin (BSA) is typically used as the reference protein in protein measurement methodologies. Despite being just a relative approach, susceptible to interference from Tris buffer, EDTA, non-ionic and cationic detergents, and carbohydrate, the method is sensitive down to roughly 10 g/ml and is arguably the most extensively used protein test.

Methodology

By severing the first proleg of the larva, haemolymph was obtained and placed in a test tube with a few thiourea crystals that had already been refrigerated. The hemolymph had been trained. The proteins from the supernatant were employed in the protein estimate after the haemolymph was centrifuged at 3000 rpm for 10 min at 4°C. The Lowry technique was used to estimate the protein. For the estimate of total soluble protein, the following chemicals are needed.

solution A : (BSA) solution (1 mg/ml). Analytical reagents: 50 ml of 2% sodium carbonate mixed with 50 ml of 0.1 N NaOH solutions. 10 ml of 1.56% $CuSO_4$ mixed with 10 ml of

2.37% Sod. Potassium tartarate solution. Analytical reagents by mixing 2 ml of (solution B) with 100 ml of (solution A).

Solution B: 2 gm Na_2CO_3 and 0.4 gm NaOH volume make up with 100 ml distilled water. Solution C: solution A (1 ml.)+B (50 ml). Solution D: 0.25 ml FCR (freshly prepared).

TABLE 1. Preparation of standard curve of protein.

T.T. No.	**Amount of sample (ml)**	**Amnt of sol. C (ml)**	**Amnt of FCR (ml)**	**OD (750 nm) (Mean value)**
1	0.0	2.5	0.25	0.000
2	0.1	2.5	0.25	0.146
3	0.2	2.5	0.25	0.206
4	0.3	2.5	0.25	0.250
5	04	2.5	0.25	0.316
6	0.5	2.5	0.25	0.386
7	0.6	2.5	0.25	0.438
8	0.7	2.5	0.25	0.478
9	0.8	2.5	0.25	0.523
10	0.9	2.5	0.25	0.597

Sample preparation:

Separate samples were generated for sericin and fibroin's electrophoretic molecular weight analysis. The hydrophilic properties of sericin. Their solubility in water varies as a result. By dissolving the silk threads in hot water kept at 80°C in a water bath, sericin was extracted. After bringing the sericin extract to room temperature, cold TCA was used to precipitate it at a final concentration of 5%. Sericin that had precipitated was centrifuged into pellets. The supernatant was thrown away while the pellets were kept. The pellet was re-suspended in an acetone/ethanol solution and centrifuged at a moderate speed to remove the lipids. Lipid-containing supernatant was once again discarded. For further estimate, the pellets were resuspended in phosphate buffer and kept at 4°C. Fibroin was extracted from silk threads using sericin-free methods. The boiling silk threads were utilised to extract the fibroin after being rinsed three times in hot water (80°C). Due to its hydrophobic nature, fibroin does not dissolve in water. By dissolving the silk thread in glacial acetic acid (10%), fibroin was recovered. Under typical circumstances, silk thread was submerged in glacial acetic acid (10%) solution and vortexes for around five minutes. In order to precipitate dissolve fibroin, cold TCA (at a final concentration of 5%) was used. Centrifugation was used to pelletize the precipitated fibroin. The supernatant was thrown away while the pellets were kept. The pellets were resuspended in an acetone/ethanol solution and centrifuged at a low speed to remove the lipids. For further estimate, the pellets were resuspended in phosphate buffer and kept at 4°C.

Discussion

Tasar silk worms are a wild species that are grown in the woodlands' natural settings. The climate has an influence on this silkworm's life cycle. There are 44 eco-races in India, which vary depending on the prevailing climate circumstances. The phenotypic and behavioural variations of the eco-races of Tasar silkworm Antheraea mylitta Drury in connection to environmental conditions have been examined by Kumari and Roy (2011a). The identification of nutritionally efficient silkworms, as well as their metabolic rate and sustained growth of their energy resources, have been the subject of some research. They came to the conclusion that the tropical Tasar silkworm might serve as a model species for the sustainable development of the rural regions' energy resources. Antheraea mylitta, a wild variant of the Tasar silkworm, was used in Roy's research of sustainable development in Santhal Parganas (Jharkhand). Roy came to the conclusion that by farming this species, both long and short term environmental management and protection of the local ecosystem will be achieved. With these goals in mind, the current study, titled "Biochemical analysis of Antheraea mylitta's haemolymph," has been carried out. In the current project, an effort was made to look into the total soluble protein (g), SDS-PAGE Analysis for molecular weight identification of sericin and fibroin of silk filament, as well as trehalose estimation using Spectrophotometric Anthrone and Phenol approach on the pupal stage of tropical wild a range of the eco-race of Antheraea mylitta D. It's noteworthy to note that total soluble protein and trehalose concentration have been found to have a strong antagonistic connection. The silk fibre is a protein that is created by silk gland cells, stored in the silk gland lumen, and then transformed into silk fibres. As a result, the silk gland is a biofactory and silk is a biomaterial with numerous uses in a variety of industries. The anterior silk gland is where the liquid silk secreted by the silkworm while spinning is funnelled through before exiting via the entrance of the spinneret. Sericin amount and kind are crucial components of the process of endowing the cocoon with special characteristics. Sericin protein is valuable due to its unique properties, which include resistance to oxidation, antimicrobial action, UV resistance, easy moisture absorption and release, and inhibition of tyrosine and kinase activity. Chemically speaking, sericin is a non-filamentous protein, but it also includes other naturally occurring impurities such lipids and waxes in organic compounds and colouring agents. Hydrophilic describes sericin. During the production of silk, it is taken out of fibroin to make it glossy and to eliminate the sericin, which as a waste product. In recent years, these seri-waste and seri-by products have been utilised as value-added goods. After degumming, 2-chain fibroin is what is left remaining. Water absorption, dyeing connection, thermo-tolerance, and UV radiation protection are all well-known properties of fibroin. Sericin from wild and domestic silkworms differs from one another in that it contains an amino acid with a non-polar side chain in the former and an amino acid with a polar side chain in the later. Alanine, glycine, and serine are present in greater amounts in fibroin. We get a tiny bit of sulphur in the fibre from cysteine residue, and this sulphur has acid side chains. The main water-insoluble protein is fibroin, which accounts for 78% of the weight of raw silk. The total soluble protein of Antheraea mylitta's haemolymph was estimated in the current study, and the value varied from 37.514 g to 38.336 g. The molecular weight of the sericin and fibroin found in the Tasar silk filament has been clearly demonstrated by the SDS-PAGE tests. The bands of sericin and fibroin differ noticeably from one another. While the molecular weight of fibroin ranged from 195 kDa to 198 kDa, that of sericin ranged from 65 kDa to 70 kDa. Antheraea mylitta's total haemolymph soluble protein concentration gradually dropped as it developed, and this could be because protein was used for silk production in the late 5 thinstars. In this work, spectrophotometric Phenol techniques were used to estimate the amount of trehalose. The total sugar using the spectrophotometric Phenol technique ranged from 0.4523 g to 0.4477 g. It's interesting to note that the concentration of trehalose and the soluble protein content of the haemolymph were closely correlated in the current investigation. Trehalose and total soluble protein content both rose concurrently during early instars, but after reaching the fourth instar, the protein level declined. Trehalose concentration, however, decreased from the first to the fifth instars, and it is now at its lowest point in the pupal stage. The current analysis provided some justification for establishing a connection between the molecular weight of sericin and fibroin and the total soluble protein trehalose. It is generally known that these insects' haemolymph serves as a warehouse for a variety of proteins and enzymes. A number of proteins in the haemolymph have been discovered using the electrophoretic technique. The silkworm Antheraea mylitta stores enough energy during the larval stage for use during the pupal and adult stages. The main and most metabolically active non-reducing disaccharide found in hemolymph is trehalose, which is produced by fat cells. The majority of the biomolecules needed for almost all of the insect's physiological functions are stored there. As a result, the alteration in haemolymph composition reflects the morphogenic and metabolic alterations occurring in insect tissue. The insect hemolymph serves a variety of purposes, including storage, transportation, and immunity. An effort has been made in the current work to look into the haemolymph of Antheraea mylitta in its standing stage. The haemolymph contains significant disaccharides called trehalose. Its fast synthesis into silk during the last larval instar of the silkworm takes place during its active biosynthesis in the fat body. The level of trehalose acts as a measure of the physiological condition of the insects since it represents the amount of carbohydrate in the body as well as the quantity of trehalose in the haemolymph. The Antheraea mylitta pupal stage is discovered to have the lowest amount of trehalose; this may be because the pupal stage is a dormant, non-feeding, diapauses condition that is encased in a cocoon. The ingestion of carbohydrates results in the lowest amount of trehalose in pupa. Trehalose, in contrast to soluble proteins, functions as an indirect element in the assessment of spinning and silk synthesis in Tasar silkworms. In the current investigation, a strong correlation between total soluble protein and trehalose level was found. Both biochemical profiles were seen at their most minimum levels during the pupal stage. The wild Tasar silkworm only grows outside in uncontrolled environments. Recent attempts to semi-domesticate this species under controlled circumstances have been attempted to prevent vagaries that interfere with its activities and the Tasar silk's characteristics. The current study will add to our understanding of how to improve the quality of Tasar silk and provide guidance for fully domesticating it. Its components include total soluble proteins, SDS-PAGE for molecular weight assessment of sericin and fibroin, and calculation of trehalose content. After modifying the silkworms' biochemical architecture, these features will be obtained.

Conclusion:

There is little information available while examining the literature on the "Biochemical analysis of haemolymph of Antherea mylitta" on total soluble proteins, quantitative and qualitative amino acid analysis, electrophoretic tests, SDS-PAGE, etc. All of these biochemical factors are important for enhancing silk production and are required for raising the calibre and output of Tasar silk. All of these details have been taken into account when conducting the current study on the "Biochemical analysis of Antheraea mylitta's haemolymph." Since the bulk of the world's poor still live in rural areas, emerging nations like India continue to place a high priority on reducing rural poverty. According to estimates from the World Bank, more than 70% of the world's impoverished reside in rural regions. The establishment of rural employment has been one of the main approaches taken thus far to address this issue. However, the agriculture industry has been dealing with a variety of issues that have restricted its ability to create new jobs in rural regions. tiny land holdings, a lack of capital and investment incentives, inadequate farm infrastructure, a tiny market, and stagnant agricultural product pricing are a few examples of these potential contributing variables. In example, the development of rural-based businesses like sericulture may be particularly successful in generating new employment possibilities and supplementary income. Being a labor-intensive rural agroindustry, this sector can actively contribute to reducing migration from rural to urban regions. The current state of the Indian sericulture sector, its trends, place in the world of sericulture, and scientific and technological advancements have all been examined for this project work. Additionally, certain important concerns have been recognised, including the sector's potential for the national economy, rural development, women's empowerment, and job creation. In an effort to increase production and silk quality, among other things, a strategy plan has been developed to support and improve India's sericulture sector. This research will aid in identifying the sericulture industry's potential, strengths, and difficulties in India so that appropriate policies and strategies for socioeconomic growth may be developed. Tasar silk worms are a wild species that are grown in the woodlands' natural settings. The climate has an influence on this silkworm's life cycle. There are 44 eco-races in India, which vary depending on the prevailing climate circumstances. They came to the conclusion that the tropical Tasar silkworm may be the ideal and model species required for the sustainable development of the rural regions' energy resources. Antheraea mylitta, a wild variant of the Tasar silkworm, was used in Roy's research of sustainable development in Santhal Parganas (Jharkhand), and he came to the conclusion that by raising this species, both short- and long-term environmental management and ecosystem conservation could be achieved [52]. With these goals in mind, the current study, titled "Biochemical analysis of Antheraea mylitta's haemolymph," has been carried out. In the current project, an effort has been made to investigate the total soluble protein (g), SDS-PAGE Analysis for molecular weight determination of sericin and fibroin of silk filament, and trehalose estimate with Spectrophotometric Anthrone and Phenol method on the pupal stage of the tropical wild variety of the eco-race of Antheraea mylitta D. It's noteworthy to note that total soluble protein and trehalose concentration have been found to have a strong antagonistic connection. 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Antheraea mylitta's total haemolymph soluble protein concentration gradually dropped as it developed, and this could be because protein was used for silk production in the late 5 thinstars. In this work, spectrophotometric Phenol techniques were employed for determining the amount of trehalose. The silkworm, also called Antheraea mylitta stores enough energy during the larval stage for use during the pupal and adult stages. The main and most metabolically active non-reducing disaccharide found in hemolymph is trehalose, which is produced by fat cells. Haemolymph is an extracellular fluid in an insect having a variety of functions, as is widely known. The majority of the biomolecules needed for almost all of the insect's physiological functions are stored there. As a result, changes in the haemolymph's composition are a reflection of the morphogenic and biochemical changes occurring in insect tissue. Haemolymph serves a variety of purposes in insects, including transport, storage, and immunity. An effort has been made in the current work to look into the haemolymph of

Antheraea mylitta in its standing stage. The haemolymph contains significant disaccharides called trehalose. Its fast synthesis into silk during the last larval instar of the silkworm takes place during its active biosynthesis in the fat body. The amount of trehalose in the haemolymph indicates the body's level of carbohydrates as well as the insects' physiological state. Trehalose levels therefore serve as a measure of the insects' well-being. he Antheraea mylitta pupal stage is discovered to have the lowest amount of trehalose; this may be because the pupal stage is a dormant, nonfeeding, diapauses condition that is encased in a cocoon. The ingestion of carbohydrates results in the lowest amount of trehalose in pupa. Trehalose concentrations rise as a result of the host plants' leaves being fed. In order to keep up with the increased physiological activity, the larval stage's faster rate of food ingestion and higher carbohydrate levels lead to a higher rate of glucose absorption through the digestive system. The pupal stage's decreased quantity of trehalose suggests that it is used more frequently to provide fuel for active. The wild Tasar silkworm only grows outside in uncontrolled environments. Recent attempts have been undertaken to semi-domesticate this species under regulated circumstances in order to prevent whims that interfere with its activity and Tasar silk's characteristics. The current study will add to our understanding of how to improve the quality of Tasar silk and provide guidance for fully domesticating it. Its components include total soluble proteins, SDS-PAGE for molecular weight assessment of sericin and fibroin, and calculation of trehalose content. These characteristics will be attained by modification of the silkworms' biochemical and structural makeup.

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